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Visual Cells and Pigments in a Demersal Fish, the Black Sea Bass (*Centropristis striata*)

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Abstract. Using a single-beam, wavelength-scanning, dichroic microspectrophotometer, we measured absolute absorbance, bleaching difference absorbance, and linear dichroism spectra from isolated retinal receptors of the black sea bass, *Centropristis striata*. We determined, among other properties, the wavelength of peak α-band absorbance (λ_{max}) of the pigment of the receptor cells. Out of well over 100 recordings, we found only 3 spectral types of visual pigment. The shortest-wavelength-absorbing type (λ_{max} = 463 ± 2 nm) was present only in single cones. Both members of the double cones contained the longest-wavelength-absorbing pigment of the three, with λ_{max} = 527 ± 5 nm. Rods were found to bear a typical rhodopsin, with λ_{max} = 498 ± 2 nm. Thus, the retina of this predatory demersal fish appears to use a set of three closely spaced visual pigments, with λ_{max} clustering about 500 ± 30 nm. This remarkable feature is discussed in relation to photic conditions in the habitat.

Introduction

Because the function of an eye is to detect light from the environment, visual systems must have evolved in harmony with the prevailing photic conditions. An adaptation of certain aspects of eye structure to an animal *habit* was clearly recognized by Schultze (1866, 1867). Being an exceptionally keen observer, he noted a correlation between the preponderance of retinal rods in the eyes of nocturnal animals, and the occurrence of numerous cones in the retinas of diurnal animals. He rightly reasoned that there is no color perception at night, that nocturnal animals are therefore adapted to dim (scotopic), black-and-white conditions, and that their vision is primarily mediated by rods. Diurnal animals, on the other hand, are mainly exposed to bright (photopic) conditions, during which color sensation is most acute, and cones must therefore be the primary mediators of color vision. Thus, Schultze's observations provided the basis for what is now known as the Duplicity Theory, which encompasses some of the most basic features of vertebrate vision.

A second link, between vision and *habitat*, was realized by Clarke (1936). He knew that the peak transmittance of pure water is in the blue part of the spectrum; and the deeper the water column to be penetrated, the more "squeezed" is the daylight spectrum about the blue peak. On the basis of this understanding, he suggested "the possibility of a shift in sensitivity of the eye of a deep water fish toward the blue end of the spectrum." Indeed, not only do fishes of the deep sea have retinas with numerous long rods, but their photosensitive pigments (rhodopsins) also have blue-shifted peak absorbance (λ_{max}) values to match the dominant wavelength of the scarce quanta available to them (Denton and Warren, 1957; Munz, 1958). From these observations stemmed the Sensitivity Hypothesis, which proposes an adaptation to the photic environment by the selection of visual pigments that render the animal's eye most sensitive to the ambient illumination.

To account for the apparent mismatch between the underwater light and the λ_{max} of the visual pigments in a number of animals, other than deep sea fish, the Contrast Hypothesis was proposed (Lythgoe, 1972). The merit of the underlying idea is the recognition that the differential scattering and selective absorption of underwater light may cause an object viewed against its background spacelight to be more visible with offset visual pigments than with matched ones. Thus, brightness contrast detection,

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in addition to sensitivity, is an important aspect of vision. So is color perception, in which λ_{max} variation of the visual pigment can play a major role. The question, at present, is not so much about the relative importance of these attributes in general, but rather their specific contribution to the evolution of the eye.

Although we still lack a unified theory of visual function, a refinement in the Sensitivity Hypothesis goes a long way toward explaining several vision-related phenomena. This refinement resulted from a series of extensive investigations (Munz and McFarland, 1973; McFarland and Munz, 1975a, b, c) in which not only the visual pigments were determined, but also the spectral radiance of natural light under many different environmental conditions was measured. It was found that the *twilight spectrum* was generally well matched by the scotopic pigments. Thus, the emerging concept was that the spectral location of *scotopic visual pigments* have been selected to enhance photosensitivity at *twilight,* for it is during this period that visual behavior is critical to survival (McFarland and Munz, 1975c).

Investigation of the *photopic visual pigments* has barely begun. Although surveys by Loew and Lythgoe (1978) and Levine and MacNichol (1979) are important contributions, providing correlations between habitat and the photopic pigments (see also Lythgoe, 1984), the development of this area of research is still in its infancy.

For the present study, we chose a day-active predatory fish that inhabits a marine environment with a fairly well delineated photic habitat. By the use of microspectrophotometry, we set out to determine the light absorbing properties of its visual pigments throughout the visible and near ultraviolet spectrum. We also made an attempt to estimate the spectral light available in the fish's environment. We report here our findings concerning the spectroscopic relationship between visual pigments and the ambient irradiance.

Materials and Methods

Experimental animals

Specimens were obtained through the courtesy of the US National Marine Fisheries Service Aquarium at Woods Hole. The fish we used were small specimens that had been trapped in Woods Hole Harbor late in the summer of 1990 (about 6 months prior to their use) and maintained in natural seawater aquaria at about 18°C (65°F). The fish were regularly fed with brine shrimp, clams, and squid, and were kept under artificial illumination (8 h on/16 h off) with some natural light filtering into the building over their tanks.

Preparation

Before use, each fish was dark-adapted for at least 1 h, and anesthetized with aerated seawater containing tricaine methanesulfonate (MS222, Sigma Chemical Company) at a concentration of 0.25 g per liter. Enucleation of the eye was performed under dim red light. The cornea, iris, and lens were removed by a cut with a sharp razor blade, transverse to the anatomical axis, about 1 mm behind the equatorial circumference of the eye, and the entire eyecup was quickly transferred to cold saline solution. With the aid of a modified, low-power dissecting microscope equipped with infrared illumination and an image converter, the retina was eased away from the back of the eye while the eyecup still submerged. From the whole retina, small pieces (about 1 mm^2) were cut, transferred to a No. 1½ coverslip, and teased apart with two pairs of fine forceps in a drop of saline solution. The fragmented retina preparation was covered with a second No. 1½ coverslip of smaller size, blotted gently along its edges, and sealed with a mixture of molten paraffin and Vaseline, as described earlier (Hárosi and MacNichol, 1974a). The saline we used here was a modified marine teleost Ringer solution (Forster and Taggart, 1950), containing 10 m*M* HEPES buffer at pH 7.3.

Spectrophotometer

The spectral measurements were carried out with the help of the dichroic microspectrophotometer (DMSP) described previously (Hárosi and MacNichol, 1974b; Hárosi, 1982, 1987). The DMSP is a computer-controlled, wavelength-scanning, single-beam photometer that records transmitted light fluxes through microscopic samples. The measuring beam is commonly adjusted to about 1×3 μm in the plane of the specimen, and its spectral purity (monochromaticity) to about 5 nm. This beam is focused by a quartz field lens onto the back aperture of the condenser through a Glen-Thompson UV polarizer and a CaF_2 photo-elastic modulator. The condenser we routinely used was a 32/0.4 Ultrafluar (Zeiss), whereas the objective was a 100/1.3 UV-F100 (Nikon) microscope objective, both of the glycerine immersion type. Due to limitations imposed by the latter objective, light detection was possible only at wavelengths greater than about 330 nm.

Spectral recording and analysis

Average and modulated light fluxes were detected with a cooled photomultiplier tube (Hamamatsu, Type R375), and photocurrents were recorded into two sets of 75 sequential memory locations as the wavelength was scanned rapidly (500 nm/s) from the short wavelength to the long wavelength end of the spectrum (275–645 nm). Corresponding signals were summed, and the memory locations thus contained numbers signifying transmitted flux amplitudes averaged over 5-nm-wide segments of the spectrum. A typical measurement included 16 background scans from a cell-free area in the preparation (reference measurement), 8–16 prebleach scans (sample measure-

ment), a 2-min exposure to actinic light provided by the measuring beam (the wavelength of which was preset to the vicinity of the expected λ_{max}) if bleaching was desired, and a 16-scan postbleach recording of the sample. The dedicated digital computer of the DMSP subsequently calculated (from the average and modulated transmitted fluxes) the average absolute absorbance (A), the bleaching difference absorbance (BD), and the linear dichroism (LD) spectra. Absorbance (optical density) is defined as $\log(T)^{-1}$, where T is transmittance. Linear dichroism is proportional to sample polarization, defined as $p = (T_{\parallel} - T_{\perp})/(T_{\parallel} + T_{\perp})$. The LD ordinate is calibrated so that a perfect analyzer would yield +1, if crossed, and −1, if parallel to the plane of the polarizer. For details of the measurement technique, the selection of spectra, and data analysis, see Hárosi (1975a, 1987).

Visual pigment characterization

Our spectroscopic description of a pigment is based on A, BD, and LD determinations from optically isolated single or overlapping multiple photoreceptor cells. Empirical evidence suggests that, in general, the three types of measurement yield three λ_{max} values that will "bracket" the "true" λ_{max} of the visual pigment. Occasionally, λ_{max} may be slightly blue-shifted (due to photoproduct absorption and excessive short-wave scattering); BD_{max} is usually red-shifted (because shortwave absorbances, which are subtracted, tend to be exaggerated); whereas LD_{max} should theoretically be close to λ_{max} (provided there is no instrumental delay between the "ac" and "dc" detection channels). Rhodopsins (based on the aldehyde of vitamin A_1, or retinal) and porphyropsins (based on the aldehyde of vitamin A_2, or 3-dehydroretinal) have several distinguishing properties *in situ:* (1) α-band half-bandwidth (HBW) value (*i.e.,* rhodopsins being narrower than porphyropsins); (2) β-band absorbance (*i.e.,* rhodopsins having relatively lower β-band absorbance than porphyropsins); (3) transverse specific density (*i.e.,* rhodopsins have higher molar extinction than porphyropsins); (4) dichroic ratio (*i.e.,* rhodopsin bearing cells show greater optical anisotropy than those with porphyropsin); and (5) absolute λ_{max} value, which is informative only beyond 570 nm (*i.e.,* no purely retinal-based pigment has ever been found with λ_{max} greater than about 570 nm).

Following Fourier smoothing of the "raw" spectra, software algorithms can determine the peak absorbance and half-bandwidth values. The α-band of a typical rhodopsin absorbance spectrum has a HBW of 4000–4100 cm^{-1}, whereas that of a typical porphyropsin is about 4800 cm^{-1}. Moreover, in both classes of visual pigment, the HBW is a function of wavelength, such that with increasing λ_{max}, the HBW progressively narrows, and with decreasing λ_{max}, it progressively broadens. We made use of these properties in our characterization of the sea bass visual pigments.

Quantal absorption of pigments vs. environmental illumination

In search of the correspondence between visual pigments and the photic environment, we analyzed models that are consistent with the premise that photoreceptors are quantum detectors with a response primarily dependent upon the *total number of quanta absorbed per unit time* by their visual pigment (rate of quantum catch), bleaching and regeneration notwithstanding. We further assumed that downward irradiance of solar origin is the primary determinant for the sea bass visual system. We made use of data available in the literature on solar irradiance and on the optical properties of natural bodies of water, and we used our own spectroscopic determinations on the photoreceptors.

We performed the following calculations. (1) Using the standard solar irradiance data of Moon (1940; Table III), by a procedure similar to that adopted by Dartnall (1975), we generated quantal irradiance values expected at sea level in quanta/s × mm^2 × nm, at 1-nm intervals (by linear interpolation). (2) The classification on optical water types and transmittance data of Jerlov (1968; Table XX) permitted the transformation of downward irradiance values to any depth. Following the suggestion of Dartnall (1975), his five oceanic water types were designated JI, JIA, JIB, JII, and JIII, and the five coastal water types as J1, J3, J5, J7, and J9. Downward irradiances were calculated for all 10 water types for depths of 10, 20, 50, 100, 150, and 200 m for 1-nm intervals. (3) The visual pigment absorbance spectra obtained at 5-nm intervals were again interpolated to 1 nm in the available range of 350–650 nm. (4) Rate of quantal absorption (quantum catch) by each visual pigment was determined for the three receptor types at six depths in ten water types. The total absorbed quantum flux density rate Q_t was, in each case, obtained by summing the products of the appropriate quantal irradiance and receptor absorptance at each nanometer of wavelength. Absorptance had the usual definition: $A(\lambda) = 1 - 10^{-D(\lambda)}$, where $D(\lambda) = D_{max} A_{rel}(\lambda)$. The peak absorbance, D_{max}, was obtained as a product $S_{\perp}$ (Table 1) and the axial pathlength through the outer segment of the receptor type containing the pigment. The average lengths observed in our video records for single cone, rod, and double cone outer segments were 9, 20, and 23 μm, respectively. $A_{rel}(\lambda)$ was derived from the normalized absorbance spectra (depicted in Fig. 5).

In an attempt to find criteria by which the correspondence between visual pigment absorptance and environmental light could be judged, we calculated the wavelengths at which 25%, 50%, and 75% of the total quantum catch occurs in each receptor type in a given photic environment. With a symbolic designation of λ_{qc50} for the 50% value, this is analogous to the λP_{50} introduced by Munz and McFarland (1973). Note, however, the differ-

Table I

Spectral data for black sea bass cones

Cone type	No. of determ	λ_{max} [nm]	HBW [cm^{-1}]	A_{max} [OD]	R	d [μm]	$S_{\perp}$ [OD/cm]
Single	6			0.03167 ± 0.0065	1.64 ± 0.19	2.9 ± 0.3	136 ± 22
A	6	463.2 ± 2.2	4638 ± 296				
LD	6	460.3 ± 2.3	4670 ± 625				
BD	3	469 ± 3.5	3737 ± 673				
A + LD	12	461.7 ± 2.7	4654 ± 467				
A + LD + BD	15	463.2 ± 4	4470 ± 616				
Best Estimate		463 ± 4	4500 ± 600				
Double	8			0.04774 ± 0.0160	1.52 ± 0.14	3.0 ± 0.4	161 ± 39
A	8	526.8 ± 5.5	3928 ± 197				
LD	8	527 ± 4.7	3728 ± 237				
BD	5	529.7 ± 4.5	3634 ± 159				
A + LD	16	526.9 ± 5	3828 ± 235				
A + LD + BD	21	527.5 ± 4.9	3782 ± 231				
Best Estimate		527 ± 5	3800 ± 200				

Abbreviations used: HBW, half-bandwidth; A_{max}, peak absorbance; OD, optical density; R, dichroic ratio; d, mean diameter of outer segment; $S_{\perp}$, specific density (transversely polarized); A, α-band of absorbance spectrum; LD, α-band of linear dichroism spectrum; BD, α-band of bleaching difference absorbance spectrum.

ence between the two: while λP_{50} is the wavelength at which 50% of all the quanta occur in the spectrum of 400–700 nm in a given photic setting, λ_{qc50} is a measure of the absorptive interaction between light and pigment throughout their available spectral range.

We also calculated total quantum catch ratios between the receptor types in the retina of this fish. Although each Q_t value is critically dependent on the axial pigment density assumed in the calculation, the Q_t ratios indicate the relative "weight" of the receptor types in the retina. As the spectral distribution of light changes with water type and with depth, the quantum catch by the receptor types vary, and the Q_t ratios may go "out of balance." Although we do not know the range of appropriate quantum catch ratios, we found them to be indicative of the "spectral match" that exists between photoreceptors and environmental light. The second criterion by which to assess the appropriateness of a visual pigment to a given environment is the difference between λ_{qc50} and the λ_{max} of the pigment in question. Again, we do not know how large this difference should be before it becomes unacceptable. Based on the experience gained in this analysis, we tentatively set the limit of acceptability at 30 nm for the difference between λ_{qc50} and λ_{max} of a pigment, and 100% for the change in Q_t ratio of two receptor types.

We made no attempt to account for the retinal distribution of the different pigments, their relative proportions, or the light collecting efficiency of the various cell types. Nor did we consider the light collection efficiency of the eye as a whole, or the losses of light that occur at the ocular media and their interfaces. The modifying effects of these factors we envisage investigating in the future as sufficiently accurate and detailed information becomes available.

Results

Although we measured well over one hundred photoreceptors, permanent records were saved from 17 single cones and 66 outer segments belonging to 39 double cones. Additionally, we recorded from 12 groups of multiple rods. In our fragmented retina preparations, the most frequently occurring cells were rods. While double cones could also be located with relative ease, single cones were less numerous. Photoreceptor morphology is illustrated in Figure 1. With these examples, we wish to make the point that double cones were variable in size and shape: the two members were practically identical in some, but quite different in others. For this reason, we simply refer to them as "double" and refrain from the use of the term "twin," even though the visual pigments in the two members were spectroscopically indistinguishable, as will be shown below.

Single cones

All single cones encountered had one type of shortwave, or blue-absorbing, visual pigment. The outer segments were transversely dichroic, and their pigment content was bleachable. Representative spectra are depicted in Figure 2. Spectral data are summarized in the upper part of Table I; note the several subgroup averages calculated for λ_{max} and HBW. The "best estimate" for each is based on the over-all average.

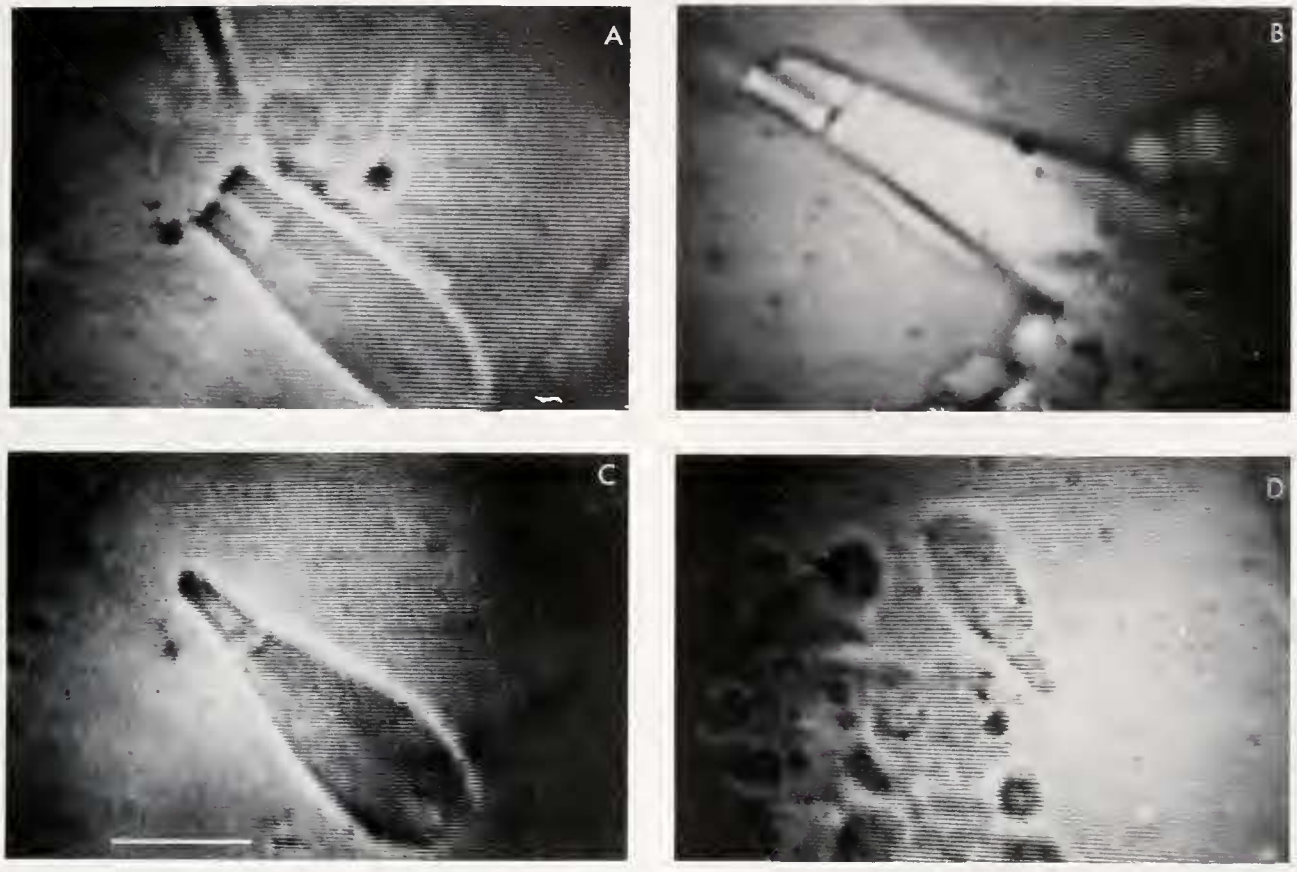

Figure 1. Black sea bass photoreceptors viewed in infrared illumination in the recording microspectrophotometer. The images were captured on video tape and subsequently photographed from a video monitor display. A. Double cone in lateral view, just below a rod outer segment. B. Double cone with unequal outer segments. C. Double cone in a rare orientation, with overlapping outer segments. D. Single cone, proximal to retinal fragments. All four panels have equal magnification and the bar length represents 10 μm.

Double cones

Every outer segment belonging to double cones was transversely dichroic due to the presence of a bleachable pigment. Representative spectra are shown in Figures 3 and 4, and spectral data are presented in the lower part of Table I. The A and BD spectra of Figure 3 were derived from one member of double cones. The spectra of Figure 4 were obtained from overlapping outer segments of double cones (see panel C of Fig. 1). Note the increased spectral absorbance in Figure 4A as compared to that in Figure 3A. The idea we illustrate here is that, when the two outer segments overlap laterally, the transversely scanned absorbance nearly doubles, as it should if the two members are equivalent. However, while the A and BD spectra increase in proportion when measured from two, instead of one member, the λ_{max} and the HBW remain virtually unchanged. This can happen only if the same pigment is contained in both members. There was no evidence for the presence of a second pigment in any of the double cones.

Rods

As is common in teleost retinas, the rods of the black sea bass are numerous; the outer segments are of variable length and slender, with a diameter of 1 μm or less. Recordings from multiple rods yielded A, BD, and LD spectra indicative of a "typical" rhodopsin. Trace B in Figure 5 was derived from such absorbance spectra. The HBW of the α-band of the rod absorbance spectra were within experimental error of the value obtainable from other rhodopsins (such as amphibian or monkey), and this pigment should, therefore, also be based on vitamin A_1. Traces A and C in Figure 5 depict the normalized absorbance spectra of the cone pigments. The two cone pigment spectra flank the rod pigment spectrum on the longwave and shortwave sides by nearly the same distance on the wavelength scale.

Dichroic ratio and transverse specific density

The algebraic relationships necessary to determine cellular dichroic ratios from the A and LD spectral mea-

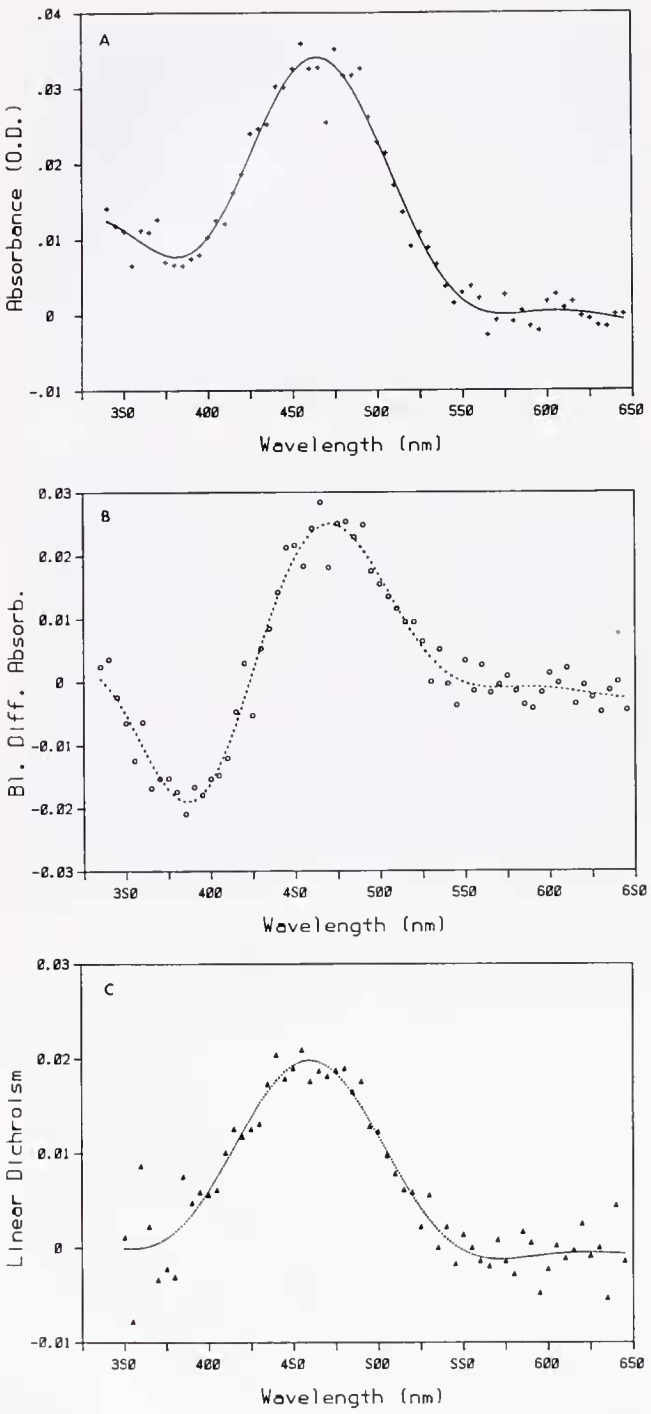

Figure 2. Absorbance, bleaching difference absorbance, and linear dichroism spectra of the visual pigment in single cones of the black sea bass. A. Average absorbance spectrum based on four single-cell recordings (+). The solid curve is the result of Fourier smoothing. Peak absorbance and half-bandwidth are 464 nm and 4430 cm^{-1}, respectively. B. Bleaching difference spectrum from one cell. Data values (○) were derived from prebleach and postbleach measurements consisting of the sum of 16 scans, each. The dashed curve is based on Fourier-smoothed data. The positive band peaks at 471 nm, with HBW = 3140 cm^{-1}; the negative band peaks at 387 nm, with HBW = 3570 cm^{-1}. C. Average linear dichroism from 3 cells (△). The dotted line, again, is the result of Fourier-filtered data with λ_{max} = 460 nm and HBW = 4460 cm^{-1}.

surements have been published previously (Hárosi, 1987). The results for the black sea bass cones are listed in Table I. The last column in Table I shows numerical values for the transverse specific density, $S_\perp$. For this determination, the transversely polarized component of the peak absorbance, $A_\perp$, is needed. The latter is derived from the average (unpolarized) peak absorbance, A, divided by factor f, which in turn depends on the dichroic ratio, R, defined as $R = A_\perp/A_\parallel$. Thus, $f = (1 + R)/2R$ and $A_\perp = A/f$. Finally, $S_\perp = A_\perp/d$, where d is the mean diameter of the compartment (Petry and Hárosi, 1990). Thus, the meaning of $S_\perp$ is peak absorbance for transversely polarized light per unit thickness (measured either in micrometers or centimeters).

Discussion

In our teased preparations obtained from various regions of the retina of the black sea bass, we found rods, double cones, and single cones. The outer segment in each of these cells contained a visual pigment characterized by a typical absorption spectrum, dichroism, and light-sensitive spectral changes (*i.e.*, bleaching). On the basis of half-bandwidth determinations, we identified the chromophore of these pigments as *retinal*. Additional evidence comes from the low β-band absorbances which we commonly observed. The results on specific density ($S_\perp$) also support this, because the cones yielded higher values of this parameter than those obtainable from cells using pigments with 3-dehydroretinal as chromophore, although not quite as high as what has been reported in cases of amphibians and monkeys. This discrepancy may be related to the results on dichroic ratio, which were also below expectation (see below).

The presence of only two cone pigments would make the black sea bass dichromatic in the traditional sense, although we have no evidence which could preclude the rods from chromatic discrimination tasks. Nevertheless, "color vision" can be supported by only two cone mechanisms, as we know from other studies on animals, as well as on humans. The existence of many vertebrates with trichromatic and even tetrachromatic *cone mechanisms* raises the question as to why this species has evolved only two. The simplest answer is, perhaps, that there was no selective pressure to have more. Given the relatively narrow spread of wavelengths at greater depths, there is probably no advantage in having more cone types, even though the eye's spectral resolution could be improved by adding more closely spaced "color channels." While vertebrate eyes would make very poor spectrographs, they nonetheless serve the bearer well. To evaluate just how well an organism is served by its eyes, we would need to know not only the lighting conditions and reflectance properties of all objects, but also the visual tasks that need to be solved in capturing food, avoiding predators, finding

mates, and continuing successful reproduction (Dartnall, 1975; Levine and MacNichol, 1979). Clearly, more knowledge is required before precise answers can be found.

Similar visual pigments have been previously reported for four other species of fish. Also using microspectrophotometry, Loew and Lythgoe (1978) investigated several species of fish from various "environmental groups." In the "moderately deep coastal group" they reported finding two cone pigments with λ_{max} of 460 and 530 nm, respectively, and a 502-nm rod pigment in two species of gurnard (*Trigla lucerna* and *Eutrigla gurnardus*). Two other species of marine fish with similar pigments were found by Levine and MacNichol (1979). These were the sea robin (*Prionotus carolinus*) and the scup (*Stenotomus versicolor*).

Photic habitat of the black sea bass

Although information on habitat is rather scanty, this fish inhabits waters within a depth range of a few meters from the surface, to 165 m. Being demersal, they are caught in large numbers in waters of 50–150 m depth. This species is mainly a bottom feeder, and prefers to be among rocks and reefs. Males have been observed to develop a bright blue color prior to spawning. Spawning involves buoyant eggs in depths ranging between 18–45 m; the larvae tend to move to inshore waters over rocky bottoms (Bigelow and Schroeder, 1953; Perlmutter, 1961; Gordon, 1977).

Relevant spectral data on habitat

There is a general dearth of information, particularly field measurements, on the photic environment of the black sea bass. The specimens we used were caught in Woods Hole Harbor, where the color of the water is green. This agrees with Clarke and Denton (1962), who reported that the maximum transparency of coastal waters can be generally found in the range of 500–600 nm. We think that type 5 of the coastal series of Jerlov (J5) would be appropriate for the optical characterization of this habitat. Because the black sea bass is a widely distributed species, ranging from southern Massachusetts to Florida and from bays and sounds to Georges Bank, it will encounter offshore oceanic waters as well. Based on Jerlov's (1968) regional classification of optical water types, the western North Atlantic is described by type IB of the oceanic series (JIB). Thus, it appears reasonable to assume, as initial conditions, that this fish's visual system needs to cope with photic habitats expected of optical waters from JIB to J5. But these *a priori* assumptions are unnecessary, for similar conclusions can be drawn from the analysis discussed below.

Correlation between downward irradiance and receptor pigments

We calculated the rate of quantum flux density absorption ("quantum catch") by the sea bass visual pigments in the downward solar irradiance (from Moon's data) transformed by the optical water types of Jerlov (see Methods for details). We also determined quantum catch (Q_t) ratios between the receptor types and the wavelength of 50% quantum catch (λ_{qc50}) for each pigment at each depth. Table II depicts some of the results. Overall, the data show good agreement between λ_{qc50} and λ_{max} of the blue pigment in ocean waters and the same for the green pigment in coastal waters. Although the absolute values of Q_t and λ_{qc50} vary for the three receptor types in the depth range of 10–200 m and across the oceanic types JI–JIII, the Q_t ratios show no drastic variations. Nor do the λ_{qc50} and λ_{max} differences. This is not true for the coastal water types. Even at 10 m depth, these parameters undergo significant changes from J1 to the other types, so that in

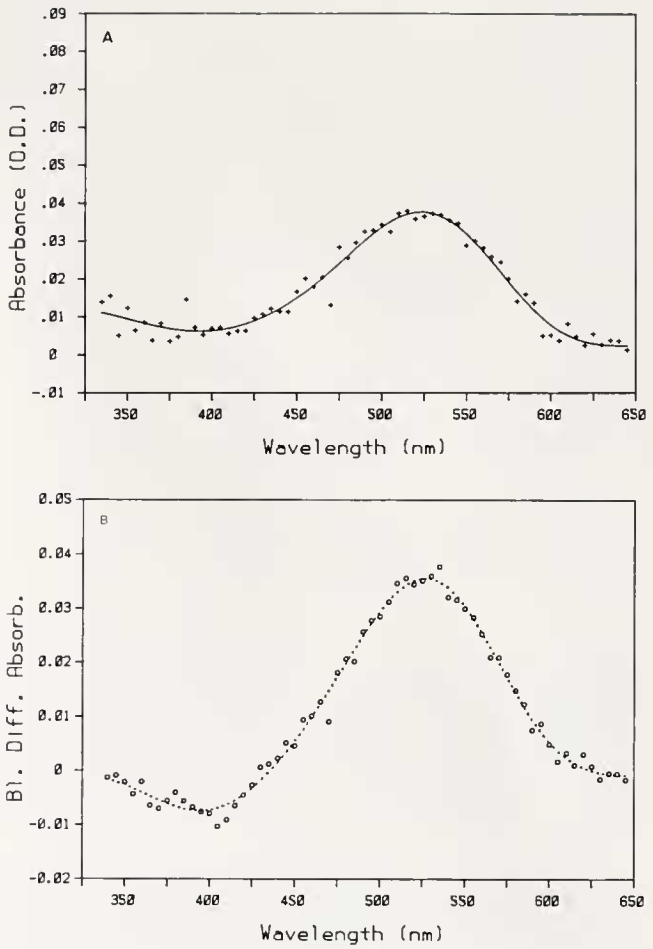

Figure 3. Absorbance and bleaching difference absorbance spectra derived from one of the outer segments of a double cone in the black sea bass. A. Average absorbance obtained in 16 scans (+). The solid curve represents the result of Fourier smoothing; λ_{max} = 524 nm, HBW = 4060 cm^{-1}. B. Average of three bleaching difference spectra, each of which is based on 16 scans (○); the dashed curve is derived from Fourier smoothing. The positive band peaks at 527 nm, with HBW = 3590 cm^{-1}, whereas the negative band peaks at 395 nm, with HBW = 4300 cm^{-1}.

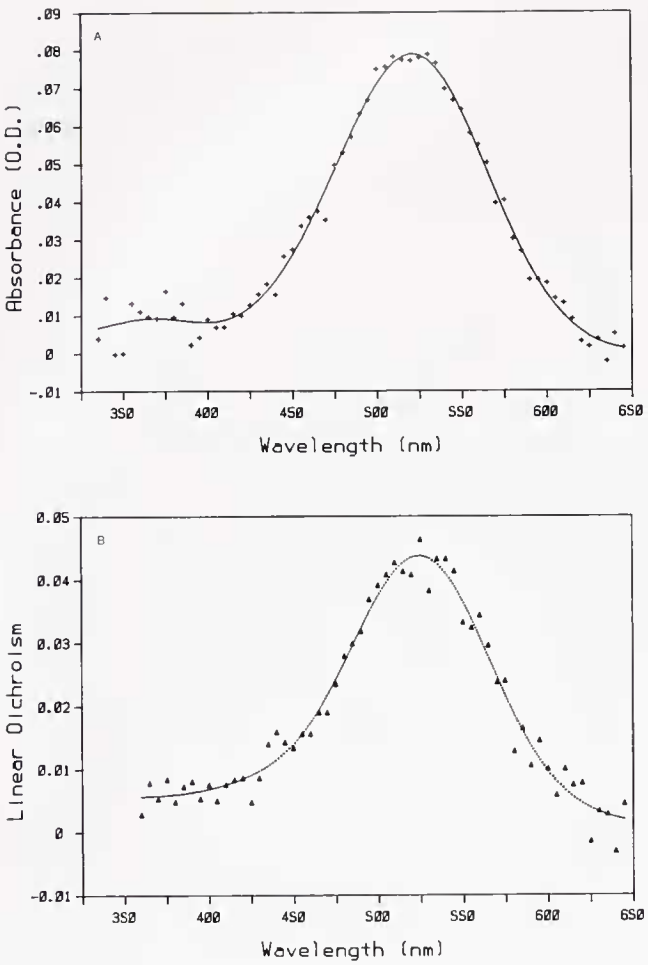

Figure 4. Absorbance and linear dichroism spectra obtained simultaneously from both outer segments (stacked one above the other) of a double cone in the black sea bass. A. Average absorbance, based on 16 scans (+); the solid curve is the product of Fourier smoothing: λ_{max} = 521 nm, HBW = 4010 cm^{-1}. B. Linear dichroism from the corresponding structure, based on 16 scans (△); the dotted curve is the outcome of Fourier filtering. The λ_{max} and the HBW are 525 nm and 3860 cm^{-1}, respectively.

J7, for example, G/B = 10.2, and in J9 it is 26.8. With increasing depth in these types of water, the sea bass visual pigments are clearly out of tune. For the J5 water type, the parameters are probably within acceptable range to depths of 20 m (see Table II). At greater depths, however, the fitness of the pigments become questionable. For instance, in J5 at 50 m depth the λ_{qc50} and λ_{max} differences are 55.6 nm, 27.5 nm, and 2.3 nm for the B, R, and G receptor types, respectively; the absolute Q_t values are down six orders of magnitude with respect to those at 10 m, and the R/B, R/G, and G/B ratios yield 5.3, 0.7, and 7.5, respectively. Although we lack firm criteria by which to interpret these numbers, they probably indicate an intolerable mismatch of the pigments to this photic habitat.

On the magnitude of the dichroic ratio in cones

Ever since the discovery of linear dichroism in laterally viewed rods by Schmidt (1938), the phenomenon has been interpreted in terms of a structural anisotropy in the outer segments of vertebrate photoreceptors. A quantitative measure of this property is the cellular dichroic ratio, R, as was defined in an earlier section. The magnitude of R is an expression of structural "order"; *i.e.*, the larger the R, the more ordered is the disposition of the visual pigment in the cell. Aside the complexities of interpretation, rhodopsin-containing rods yield larger R values than porphyropsin-bearing rods (Hárosi, 1975b). Furthermore, cone dichroic ratios are always smaller than those obtainable from rods, regardless of pigment class. In published accounts, for example, goldfish (Hárosi and MacNichol, 1974a), Japanese dace (Hárosi and Hashimoto, 1983), and carp (Hawryshyn and Hárosi, 1991) yielded mostly values with $R \geq 2$. In comparison, the average values we obtained for the black sea bass were $R_{double} = 1.52 \pm 0.14$ (n = 8) and $R_{single} = 1.64 \pm 0.19$ (n = 6) (see Table I). Although in one instance R = 1.9 was found in a single cone, we conclude that the values are rather smaller than they ought to be. Obviating the trivial interpretation of these results as instrumental artifact, there is the possibility that our experimental specimens were subnormal in their photoreceptors. The notion we entertain here is that these fish, although appearing quite healthy, nonetheless have suffered *subtle structural damage* in their retinal receptors

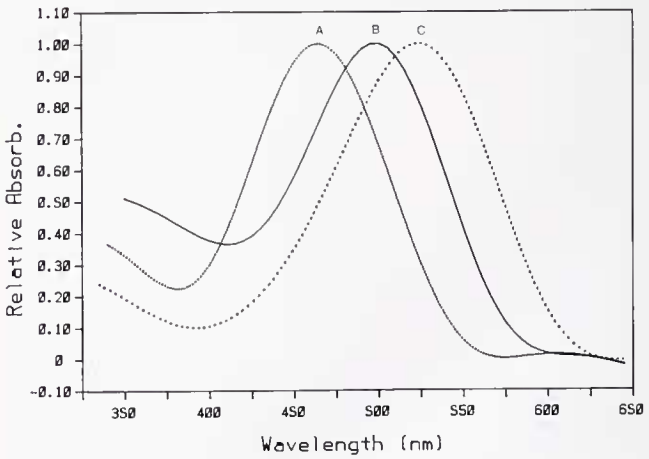

Figure 5. Comparison of relative absorbance spectra determined from the three visual pigments present in the black sea bass. Each curve was obtained from an experimental absorbance spectrum by dividing the data set by its peak value. The dotted curve (A) is based on Figure 1A. The solid curve (B) was derived from two sets of multiple rod absorbance measurements, each consisting of 16 scans. The dashed curve (C) was replotted from the spectrum of Figure 2A. The λ_{max} and the HBW of these spectra are: for single cone, 464 nm, 4430 cm^{-1}; for rod, 498 nm, 4260 cm^{-1}; for double cone, 524 nm, 4060 cm^{-1}, respectively. Note that the two outer segments of double cones contained visual pigments that were spectroscopically indistinguishable from one another.

Table II

Absorbed quantum flux density rates by black sea bass visual pigments, Q_t, in 10^{12} quanta/s × mm² (integrated in 1-nm steps from 350–650 nm) as a function of depth

Depth [m]	Single cone B, λ_{max} = 463 nm		Rod R, λ_{max} = 498 nm		Double cone G, λ_{max} = 527 nm		Quantum catch ratios		
	Q_t	λ_{qc50} [nm]	Q_t	λ_{qc50} [nm]	Q_t	λ_{qc50} [nm]	R/B	R/G	G/B
For oceanic water type JIB:									
10	40.08	472.0	111.49	491.0	122.32	509.2	2.782	.911	3.052
20	27.15	470.0	71.22	486.8	73.18	501.3	2.624	.973	2.696
50	8.762	468.4	20.49	478.8	19.04	487.9	2.338	1.076	2.172
100	1.426	466.1	3.022	472.9	2.630	477.7	2.119	1.149	1.844
150	.2435	465.1	.4901	470.1	.4148	473.5	2.013	1.182	1.704
200	.0427	464.5	.0835	468.6	.0696	471.2	1.953	1.199	1.629
For coastal water type J5:									
10	1.106	499.1	4.429	516.7	6.248	529.1	4.005	.709	5.651
20	.0319	507.9	.1462	521.5	.2088	529.8	4.580	.700	6.541

Notes:

(1) B and G are blue- and green-absorbing visual pigments, while R stands for rhodopsin of the rod.

(2) Absorbed quantum flux density was obtained from absolute *absorptance* of a pigment by the relation $A(\lambda) = 1 - 10^{-D(\lambda)}$, where $D(\lambda)$ is optical density at a given wavelength. $D(\lambda)$ was derived from the relative absorbance values (spectra shown in Fig. 5) multiplied by the peak axial absorbances of the pigments *in situ*. Actual peak absorbances used for the single cone, rod, and double cone pigments were 0.12, 0.32, and 0.37, respectively.

(3) JIB and J5 correspond, respectively, to the optical water types IB and 5 of Jerlov (1968).

(4) λ_{qc50} is the wavelength at which 50% of the "total quantum catch" is attained by a visual pigment in a given photic environment.

due to the artificial illumination (and possibly from nutritional deficiency) while in captivity. In view of a number of recent studies of light damage conducted primarily on mammals, the idea has rational basis and appears worthy of further investigation.

Summary

By determining the visual pigments in the retina of the black sea bass and analyzing the expected quantum catch by the photoreceptor types, we obtained indications for the preferred photic habitat. We used two criteria to assess the fitness of a set of visual pigments to ambient light: (1) ratio of quantum catches by the receptor types; and (2) the deviation of the wavelength at 50% quantum catch from the λ_{max} of each pigment in a given photic environment. Indications were that the black sea bass has a balanced set of three pigments to match the downward irradiance spectrum of clear ocean water to depths of 200 m. However, for coastal waters the fit is limited to the optical types J1, J3, and J5, and the last type to only a depth of about 20 m. Water types J7 and J9 are expected to be unsuitable to the black sea bass at all but the shallowest of depths. *This suggests that the λ_{max} of visual pigments in a multi-pigmented system are selected on the basis of the photic interaction between environmental light and the pigment spectrum to produce a balanced quantum catch in the receptor types.* Whether this is a general rule, or there are other criteria by which one λ_{max} is preferred over another, are questions to be settled by future investigations.

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